PRELIMINARY STUDIES ON THE EFFECTS OF VARYING LEVELS OF AERATION ON THE GROWTH AND PHYSIOLOGICAL PROCESSES OF CULTURED *CLARIAS GARIEPINUS* FINGERLINGS

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Abstract

To determine the aeration regime and the resultant dissolved oxygen input that imposes the least physiological stress on homeostatic mechanisms *C. gariepinus* fingerlings, were culture in the laboratory under 24 hours of continuous aeration, inputing a mean of 8.2 mg/l of 0_2; 12 hours of aeration (mean of 4.6mg0/l $_2$); and zero-aeration (mean of 1.2 mg0/l $_2$). Survival rate, growth rate and mean weight gain per day were highest in the 4.6 mg0/l_2 culture whereas, the other two cultures were characterized by higher mortality rates and insignificant growth rates. The haematological profile analyzed showed varying degrees of changes in the blood parameters of the fish cultured under varying levels of aeration and their inputed dissolved oxygen levels.

Introduction

The economic viability of many intensive fish culture operation depends on the fine balance between maximum utilization of water and food supplies and the avoidance of stress-related problems (Pickering *et al*, 1982). The development of appropriate management procedures in aquacultural practises has come to include monitoring of stress responses of the cultivable species as an integral part of this development, as it provides information on the health status of the animals (Iwama, *et al* 1995; Ruane, *et al* 2002).

Oxygen is an important water quality factor in fish production, and enters the body in most post-larval fishes through the gill surface (Van Damm 1995). When the annual statistics about fish mortality in natural lentic and lotic waters , in ponds under fish culture, in enclosure afloat, in lakes and in closed systems undertaking intensive culture with water recycling, this brings to the fore the predominance of deaths due to oxygen deficiency. These deaths due to deficiency in dissolved oxygen content of the water are in most cases caused by the introduction of substances of organic origin capable of putrefaction. Besides death caused by severe hypoxia and anoxia, decreased oxygen availability may limit the distribution of fish species by reducing the energy available for locomotion, growth and reproduction (Brett and Grooves 1979).

This study is conducted with regards to the alternative air-breathing modes of this species (*Clarias gariepinus*) in the area of aquatic surface breathing, while excluding the behavioural response to hypoxia that has been the use of avoidance as a measure of the change of habitat induced by reduced oxygen availability (Doudoroff and Shumway, (1970). The main objective of this study is to determine the level of aeration with the resultant dissolved oxygen input, that will less prove stressful to cultured *C. gariepinus* fingerlings.

Materials and Methods

Clarias gariepinus fingerlings used for this work were harvested from Monai shore of Kainji Lake. They were transported to NIFFR Natural Fish Food production centre for the acclimatization process which lasted for two months. In the acclimatization tanks, they were fed on 40% crude protein pelleted artificial feed, and were routinely examined for pathological disorders. Mortality during the acclimation process was less than 2% and this was attributed mainly to stress during capture. The experimental aquaria were arranged in three sets and duplicated twice. The first set had all the experimental variables maintained at optimal level, including the test variables (aeration with dissolved oxygen as the most important probe component). This was done by maintaining a constant supply of air by the use of compressor operated, electricity powered air-pumps, working continuously for 24 hours daily throughout the duration of the experiment. The second set had all the experimental variables maintained at optional level except the test variable (degree of aeration). The level of aeration was lowered to 12 hours daily (from 6a.m to 6p.m). In the third set of experiment, all the experimental variables were optimally maintained, but there was zero aeration.

Uniform duration of water change for the three experiments was at three days interval, and they were fed twice daily at 2% of body weight on specially formulated catfish grow-out feed. That experiments ran through a period of 12 weeks before they were terminated. Water quality parameters were regularly monitored and analyzed before every water change in each aquarium for conductivity total dissolved solids suspended solids ammonia (NH_3) Nitrate ($N0_3$) Carbon dioxide ($C0_2$) Sulphate (S_2) Carbonate ($C0_3$) Phosphate according to APHA (1985).

The membrane filter counting technique was used in this work Cultures were made on Nutrient agar, MacCkonkey agar, Chocolate agar and Blood agar. A $^1/_{100}$ dilution of the oxidized sample was ascetically made before the filtration and incubated aerobically for 24 hours and the isolates identified. Colonial count was made by counting the number of colony growth for each isolate on the membrane filter, and biochemical tests carried out for identification. Sub-cultureswere made where necessary for better identification. Counting, was made for colonies with the same Blood samples were collected using the cardiopuncture method with a long glass capillary of 200 mm whose inner surface is lined with a fine film of heparin. Blood appears under mild hydrostatic pressure in pulses into the capillary. Erythrocyte count was determined in heparinized blood diluted by the Hayem solution at a ratio of 1:200. The red blood cells were counted in 20 rectangles, regularly distributed over the whole lattice of the counting cell at 200. fold magnification. The resultant counted amount of erythrocytes was then reduced 100 times and the resultant value is the number of erythrocytes in $T.1^{-1}$ (T tera 10^{12}) (Svobodova, *et al* 1991). The differential leukocyte counts were made from air-dried smears fixed in methanol for 30 minutes and stained with Wrights Giesma (Humason 1972).

Haemoglobin content of the blood was determined by the cyanomethamoglobin method, using Van Kampen and Zijistra, transformation solution. The transformation is rapid, and the data

Was read out from Fisher's haemophotometer (Model 55) after 3 minutes, Catfish blood gives consistently lower reading with the cyanomethamagbobin technique (Scott and Rogers 1981), thus the catfish blood correction factor of Harsen (1964) was employed, and the result obtained cross checked with that obtained by using Mercekotest diagnostic kit.

Blood for Packed Cell Volume (PCV) determination was placed in two microhaematocrit tubes. The tubes are put into the centrifuge (type 316, Speed 14,000 r.p.m) and centrifuged for 3 minutes. After centrifugation, the haematocrit percentage was directly read on the haematocrit meter which is part of the haematocrit centrifuge set. The percent value obtained in this way is multiplied by coefficient 0.01 and the resultant value obtained is the PCV. The basic erythrocyte data and the morphologic indices of Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated after Weinberg *et al* (1972).

Result and Discussions

From Table 1, the 24-H aeration experimental culture medium was observed to be non-slimy in-between the periods of water change, that of the 12-H was slimy and the zero-aeration experiment very slimy. This could be an indication of the concentration of superficial mucous cells in the epidermis resulting in higher degree of mulcification. Information on bacterial microflora associated with warm tropical aquaculture is scanty as well as on larval health and growth. In Table 2, the dominant microbial population in the three culture media were *Escheri coli*, *Streptococcus spp* and non-specific *coliforms*. The role of these bacterial populations in the culture media is not very clear and may need to be studied further. Sugita *et al* (1988) postulated that some bacteria existing in culture water may be commensals populations with the fish producing a unique physical and chemical environment suitable for their development. However, some species of bacteria are potential pathogens capable of causing unacceptable losses in fisheries (Schachte, 1970).

Fish need oxygen for aerobic generation of energy for body maintenance, locomotion, feeding and biosynthesis (Van Damme and Paul, 1995). *Clarias gariepinus* is a facultative air-breather i.e. breathes air at all levels of dissolved oxygen but can survive without air-breathing. In this study, dissolved oxygen via aeration is supplied the fingerlings in excess of the nomoxic condition at 8.2mg/l. Bevan and Kramer (1987) showed that, when prevented from air-breathing at normoxia *C. macrocephalus* increased their water-breathing by over 80%. Thus, the effect of surface access on survival time tends to corroborate the suggestion from this studies of the effects of oxygen concentration that survival time is affected by the rate of gill ventilation.

Table 3 shows the blood cell counts. The erythrocyte numbers, the hemoglobin content and the hematocrit value of the zero-aeration experiment showed statistically significant ($P<0.05$) increases over the 24-hour aeration experiment. Red cell numbers and haemoglobin concentrations reflect the oxygen carrying capacity and efficiency of the blood (Breazile *et al*, 1982). Whereas the total oxygen demand of fish is computed as the sum of routine metabolism, feeding metabolism and energy for biosynthesis and feeding rate is limited to a level where total oxygen demand does not exceed the potential supply (Van Damme & Pauly, 1995).

Higher blood glucose levels were recorded in the 24-H aeration with mean of 2.5 nmols/L and the 12-H aeration with mean of 2.1 nmols/l. When subjected to statistical analysis, these were not significantly different from the zero-aeration mean (1.9 nmols/litre). Hyperglycaemia is perhaps the most frequently used indicator of stress responses in the teleost fish, yet its magnitude and duration are strongly dependent upon the strain and nutritional status of the fish, and on environmental temperature (Pickering et al, 1984).The zero-hour aeration experiment had higher numbers of leucocytes (white cells) with mean (7.66) higher than while 12H was higher than 24H with mean of 6.98. However, these changes do not show statistical differences. Changes in the concentration of circulating white cells are more consistent. It was also observed that in some species, however, that the leucocytic responses to stress may be more complex e.g. in *Fundulus heteroclitus* where the stress of acute cold shock produces a complex lymphopoenia/lymphocytosis sequence (Slicher 2002).In conclusion, numerical values for this species under the experimental condition as determined in this study were specific to the acclimation processes, test conditions and experimental temperatures and may or may not likely remain constant under different more complex field conditions.

TABLE 1:

VISUAL AND SUBJECTIVE OBSERVATIONS OF THE CULTURE WATER

EXPERIMENT	REPLICATE	TYPE OF MATTER	COLOUR	CONSISTENCY
24 HRS AERATION	A_1	*	0	Ó
	A_2	*	00	Ó
	A_3	*	0	Ó
12 HRS AERATION	B_1	*	000	ÓÓ
	B_2	*	00	ÓÓ
	B_3	* *	000	ÓÓ
0-HR AERATION	C_1	* *	0000	ÓÓÓ
	C_2	* *	0000	ÓÓÓ
	C_3	* *	0000	ÓÓÓ

KEY

Matter

* **Non - particulate** Ó = Non slimmy

* * **Particulate** Ó Ó = Slimmy

Ó Ó Ó = Very slimmy

0 = Clear

0 0 = Creamy

0 0 0 = Light Brown

0 0 0 0 = Greenish

TABLE 2:

TOTAL VIABLE BACTERIAL COUNT IN THE CULTURE WATER MEDIA

(10^2 cfu/ml)

EXPERIMENT	REPLICATE	ESCHERICOLI	STREPTOCOCUS SPP	NON-SPECIFIC COLIFORMS	MICROCOCCUS SPP
24 HRS AERATION	A_1	40	21	23	-
	A_2	42	21	21	-
	A_3	38	18	31	-
	X ±S.D	**40 ± 1.63**	**20± 1.15**	**25± 4.0**	**-**
12 HRS AERATION	B_1	14	20	3	-
	B_2	18	22	1	-
	B_3	24	20	-	-
	X ±S.D	**18.66 ± 4.14**	**20.6 ± 1.58**	**1.3 ± 0.9**	**-**
0-HR AERATION	C_1	10	40	-	-
	C_2	16	29	-	-
	C_3	21	18	-	-
	X ±S.D	**15.66 ± 4.4**	**29 ± 9.0**	**-**	**-**

TABLE 3:

ANALYTICAL (PROFILE) OF BLOOD SAMPLE OF FISH CULTURED UNDER DIFF. AERATION REGIMES

					%	%	%	%	%	(nmo1/l)
EXPERIMENT	REPLICATES	(g/100ml) Hb	RBC	WBC	NEUTROP-TICS	LYMPHO-CYTES	MONO-CYLES	EOSINO-PHILS	BASOPHILS	BLOOD G/UCOSE
24 HRS AERATION	A_1	10.22	10.63	7.20	60	44	3	0	-	3.40
	A_2	9.20	11.44	6.10	57	40	2	0	-	2.00
	A_3	10.40	12.0	7.65	58	42	1	0	-	2.120
	x ± S.D	**9.4 ± 0.5**	**11.35**	**6.98**	**58.33**	**42**	**2**	**0**		**2.5**
12 HRS AERATION	B_1	11.10	13.05	8.45	50	45	5	0	1	3.0
	B_2	10.70	12.95	7.10	42	41	5	1	1	1.60
	B_3	11.40	12.60	6.80	63	42	3	0	-	1.80
	x + S.D	**11.06 ± 0.2**	**12.86**	**7.45**	**51.66**	**42.66**	**4.33**	**0.3**	**0.6**	**2.1**
0-HR AERATION	C_1	12.24	14.65	7.10	65	43	7	0	2	2.40
	C_2	13.05	13.78	7.70	63	44	12	2	2	2.20
	C_3	13.30	13.27	8.20	65	4.6	4	0	1	1.05
	x + S.D	**12.86 ± 0.19**	**13.9**	**7.66**	**64.33**	**44.33**	**7.66**	**0.6**	**1.6**	**1.9**

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